An updated review of routine bacterial surveillance of platelet concentrates

Scientific Seminar on Transfusion Medicine jointly organized by the HK Red Cross Blood Transfusion Service and the HK Association of Blood Transfusion and Haematology
22 November 2003

Background

- Transfusion transmitted infection vs blood safety in a climate of public confidence.
- Technologies have substantially improved blood viral safety.
- Bacterial sepsis is still a significant and universal problem - can result in significant rapid onset morbidities and even mortalities.
Frequency of Transfusion-Related Fatalities

FDA Data: 1990-1998

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemolysis</td>
<td>161 (50%)</td>
</tr>
<tr>
<td>Bacterial contamination</td>
<td>46 (10%)</td>
</tr>
<tr>
<td>TRALI</td>
<td>29 (9%)</td>
</tr>
<tr>
<td>Non-bacterial infections</td>
<td>23 (7%)</td>
</tr>
<tr>
<td>Transfusion-associated GvHD</td>
<td>18 (6%)</td>
</tr>
</tbody>
</table>

J-H Lee, MD, CBER, FDA, 9/24/99

Death Blamed on Contaminated Blood!

Japan

The Asahi Shimbun, Wednesday, September 3, 2003

- Platelets, *Streptococcus pneumoniae*
- Male, died 9 hours after transfusion
- Same species ‘also turned up in a different sample of the same donor’
Residual risks of TTI

Transmission risk, per unit

- Bacterial Contamination (platelets): 1:1000
- Clinical Sepsis (platelets): 1:10,000
- Septic Fatalities (platelets): 1:100,000
- HIV: 1:100
- HBV: 1:1,000
- HCV: 1:10,000

Years


Goodnough LT et al. NEJM 1999;341:126-7

Hong Kong Red Cross Blood Transfusion Service, Hospital Authority

Residual risks of TTI

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Years


Goodnough LT et al. NEJM 1999;341:126-7

Hong Kong Red Cross Blood Transfusion Service, Hospital Authority
From a local clinical study, the risk of septic transfusion per unit of platelet concentrate is 1 in 2,000 (0.046%) among the BMT recipients

(A prospective study of symptomatic bacteremia following platelet transfusion and of its management Chiu EKW et al. Transfusion 1994;34:950)

Contamination sources

• Endogenous

• Exogenous

• Unknown
Endogenous

- Osteomyelitis
  - Staphylococcus
  - S. cholerasuis

- Teeth
  - Staphylococcus sp.
  - Streptococcus viridans
  - Serratia liquefaciens

- Guts
  - Yersinia enterocolitica
  - Salmonella sp.
  - Campylobacter sp.

Donor Screening

Exogenous

Normal Skin

- Staphylococcus epidermidis
- Staphylococcus aureus
- Diphteroids sp.
- Micrococcus sp.
- Pseudomonas sp.
- Bacillus cereus
- Propionibacterium acnes
- Flavobacterium sp.
**Exogenous**

- Venipuncture standardization - Most important
- Contamination present in multiple puncture sites
- Dermic embolus - skin, follicles, sebaceous glands

**Collection tubes** ⇒ *Serratia marcescens*

**Non-sterile saline** ⇒ Manipulation

**Water-bath** - (*Burkholderia cepacia, P. aeruginosa, P. fluorescens*)
Exogenous

- Contaminated bags - Scandinavia - *S. marcescens*
- Sterile connecting device - 1% failure (*Aubuchon et cols*)
- Pinholes

Strategies to Decrease the Risk

- Donor Screening
- Improved Disinfection
- Diversion of the 1st Part of the Whole Blood Donation
- Bacterial detection
- Pathogen inactivation/reduction
Impact of donor skin preparation on the risk of bacterial contamination

CK Lee, PL Ho*, NK Chan, A Mak, J Hong, CK Lin. Hong Kong Red Cross Blood Transfusion Service and *Department of Microbiology, Queen Mary Hospital, the University of Hong Kong

Disinfectant used

- **Method A**
  0.5% cetrimide/0.05% chlorhexidine solution followed by 70% alcohol (contact time for each was approximately 30 seconds)

- **Method B**
  10% povidone-iodine followed by 70% alcohol (contact time for each was approximately 30 seconds)
Results

<table>
<thead>
<tr>
<th></th>
<th>Cetrimide/chlorhexidine, then alcohol</th>
<th>Povidone–iodine, then alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of platelet concentrates examined</td>
<td>86558</td>
<td>86123</td>
</tr>
<tr>
<td>No. of platelet concentrates with bacterial contamination</td>
<td>62</td>
<td>36</td>
</tr>
<tr>
<td>Rates of bacterial contamination of platelet concentrates*</td>
<td>0.072%</td>
<td>0.042%</td>
</tr>
</tbody>
</table>

*P = 0.0093 by Chi–square test; relative risk reduction 41.7%.
Results: Micro-organisms identified

<table>
<thead>
<tr>
<th></th>
<th>Cetrime/chlorhexidine, then alcohol</th>
<th>Povidone–iodine, then alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P = 0.7497 by Chi-square test</strong></td>
<td><strong>Bacillus species</strong> 24 (38.7%)</td>
<td>16 (44.4%)</td>
</tr>
<tr>
<td></td>
<td><strong>Coagulase negative staphylococcus</strong> 26 (41.9%)</td>
<td>15 (41.7%)</td>
</tr>
<tr>
<td>Others: included Lancefield Group G Streptococci, Propionibacterium species, Diphtheroid bacilli, Proteus mirabilis</td>
<td>12 (19.4%)</td>
<td>5 (13.9%)</td>
</tr>
</tbody>
</table>

Positive rate of bacterial surveillance test

Implementation of iodine as skin disinfectant
Conclusion

- Povidone–iodine and alcohol is more effective than cetrimide/ chorhexidine and alcohol in prevention of venepuncture–associated contamination of platelet concentrates by skin flora.
- However, it cannot completely eliminate contamination.

Prevention: diversion of 1st 10 cc.
### Reduction of bacteria after diversion of first aliquot of blood

<table>
<thead>
<tr>
<th></th>
<th>Standard whole blood collection</th>
<th>Diversion of the 1st 10 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donations tested</td>
<td>18,257</td>
<td>7,115</td>
</tr>
<tr>
<td>Prevalence</td>
<td>0.34%</td>
<td>0.21%</td>
</tr>
<tr>
<td>Confidence interval</td>
<td>0.25-0.44</td>
<td>0.12-0.35</td>
</tr>
</tbody>
</table>

Reduced numbers of Staphylococci.
De Korte, Marcelis et al. 2000

### CONCLUSIONS

- Prevalence of bacterial contamination in whole blood collections can be reduced significantly by removal of first amount of blood:  
  \[0.34\% \xrightarrow{} 0.21\%\]
- The theoretical contamination risk of pooled platelet concentrates composed out of 5 single donor units is still considerable: ~ 1%!
- Screening platelets is recommended.
5.1.5.1 The blood bank or transfusion service shall have methods to limit and detect bacterial contamination in all platelet components.
Standard 5.6.2 applies. [Arm Prep]

5.1.5.1 Standard 5.1.5.1 shall be implemented by March 1, 2004.

CAP

College of American Pathologist’s has included Bacterial contamination of Platelets in their Accreditation Checklist (December, 2002):

“TRM.44955 Phase 1 Does the laboratory have a system to detect the presence of bacteria in platelet components?”
Reduction of platelet transfusion-associated sepsis by short-term bacterial culture  

- 26,210 whole-blood-derived platelet components were tested by aerobic bacterial culture on day 2.
- 14 (0.053%) platelet units were found to be contaminated.
- nine of the associated red cell units and 4 fresh-frozen plasma units grew the same organisms on culture.
- Risk of bacterial contamination: 1:1872

HK Red Cross Blood Transfusion Service implemented a program of pre-release bacterial surveillance for platelet concentrates since January 1998
A total of 543,819 units of platelets have been cultured (up to end of January 2003)

Test Method

Sample

- Platelet concentrates prepared from whole blood units which have been stored at RT for 6 to 24 hours,
- 1 to 1.5 ml from each platelet unit on D2,
- Samples from 5 units pooled into one for culture.
Test Method

Reagent & Equipment

- BacT/Alert aerobic culture bottles
- BacT/Alert Automated Microbial Detection System

Procedure

- Inoculate the pooled samples into aerobic culture bottles,
- Incubate for 24 hours at 35°C,
- If negative, platelets can be issued,
- Further incubate for 24 hours.
Platelet concentrates on Day 2 of collection

 Strip and mix the PRP in tubing and bag
Seal the tubing segment at the required length

Fasten the 5 tubing segments together
Assign an accession number to each member of the platelet bag and tubing bundle in one pool.

Cut the segments out.
Label the aerobic culture bottle with the same accession number

Associate the 5 donation numbers with the accession number of the bottle
Fit the bundle of segment into a rack
Immerse the segment bundles with the rack into alcohol for 10 min for sterilization.
Prepare the aerobic culture bottle

Aspirate sample from tubing segments with syringe
Inoculate into culture bottle
Register bottle for loading into the detection system

Load the bottle into the detection system
Initial report printed after first 24 hours of incubation

Release platelet if result is negative after the first 24 hours
Final report printed after full 48 hours incubation

Initial positive rate

0.47% per pool
Follow up of initial positive samples

**Culture positive alarm**

*Perform Gram’s stain on positive bottle*

- Reload bottle, continue monitoring
- **Yes**: Bacteria observed in Gram’s smear
  - 1. Blood Product Retrieve and Recall (if required),
  - 2. Perform culture for individual platelet (sample direct from bag),
  - 3. Send repeatable culture-positive units to reference lab for identification.
Confirmed Positive Rate

- Per pool: 0.25% (53% of the initial positive pools)
- Per unit of platelet: 0.0519% (1/1927 units)

Bacterial surveillance positive rate

细菌监测实验阳性率
Confirmed positive cases

Among the 282 confirmed positive cases:
- Bacillus spp: 151 (53.5%)
- Coag-neg Staph: 93 (33%)
- Misc Gram positive org: 14 (5%)
- Misc Gram negative org: 24 (8.5%)

Misc Gram +ve organisms include:
- Lancefield Group G streptococci
- Non-enterococcal Group D streptococci
- Micrococcus spp
Misc Gram –ve organisms include:

- E coli
- Klebsiella oxytoca
- Proteus mirabilis
- Salmonella group D
- Flavobacterium spp
- Haemophilus influenzae

Breakdown of positive samples by detection time:

- 0 - 24 hours: 93%
- > 24 - 48 hours: 7%
Recall of platelets from hospitals

- 173 units recalled because of initial positive
- 6 (3.5%) confirmed positive
- 3 (50%) due to Bacillus
- 3 (50%) due to CNS
Conclusion

1. Test simple and not expensive;

2. There has been no documented report of septic platelet transfusion since introduction of BST in 1998 in HK;

3. Effective in eliminating the risk of sepsis due to bacterial contamination

Disadvantage

1. Platelets are normally released at Day 3 of collection ⇒ shorten the available shelf life;

2. One positive platelet will cause wastage of other platelets in the pool.
Residual Risk of Bacterial Contamination Of Platelet Concentrates At The End Of Shelf Life (Day 5 & 7) After Routine Bacterial Surveillance At Day 2

Objectives of study

- To quantify the residual risk of bacterial contamination of platelet concentrates at the end of their shelf life (5 and 7 days) by culture method after routine bacterial surveillance testing (BST) at day 2,

- To identify the nature of bacteria involved.
Protocol

<table>
<thead>
<tr>
<th>Day</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>Blood collection</td>
</tr>
<tr>
<td>Day 1</td>
<td>Component preparation</td>
</tr>
<tr>
<td>Day 2</td>
<td>Bacterial culture for 24 hours (BST)</td>
</tr>
<tr>
<td>Day 3</td>
<td>Release of BST negative PC, continue to monitor BST for another 24 hours</td>
</tr>
<tr>
<td>Day 4</td>
<td>BST completed</td>
</tr>
<tr>
<td>Day 5</td>
<td>PC expires</td>
</tr>
<tr>
<td></td>
<td>50% expired PC → bacterial culture on expiry</td>
</tr>
<tr>
<td></td>
<td>50% expired PC → bacterial culture on Day 7</td>
</tr>
</tbody>
</table>

Results(1)

<table>
<thead>
<tr>
<th></th>
<th>Group A (PC stored for 5D)</th>
<th>Group B (PC stored for 7D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of samples studied</td>
<td>3010</td>
<td>3010</td>
</tr>
<tr>
<td>No. of initial but not confirmed positive cultures</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total no. of confirmed positive cultures</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Overall positive rate</td>
<td>0.133%</td>
<td>0.133%</td>
</tr>
</tbody>
</table>
### Results (2)

<table>
<thead>
<tr>
<th>Case</th>
<th>Gram Stain</th>
<th>Bacteria identified</th>
<th>Time to detect</th>
<th>Culture of associated blood products</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>S731</td>
<td>+ve  <em>Coagulase Negative Staphylococcus</em></td>
<td>4.2 hours</td>
<td>RC (-), FFP (-)</td>
</tr>
<tr>
<td>SX290</td>
<td>-ve</td>
<td><em>Propionibacterium acnes</em></td>
<td>6.6 days</td>
<td>RC (+), FFP (-)</td>
</tr>
<tr>
<td>S1222</td>
<td>-ve</td>
<td><em>Propionibacterium acnes</em></td>
<td>5.3 days</td>
<td>RC (+), FFP (-)</td>
</tr>
<tr>
<td>SX1046</td>
<td>-ve</td>
<td><em>Propionibacterium acnes</em></td>
<td>5.8 days</td>
<td>RC (+), FFP (-)</td>
</tr>
</tbody>
</table>

### Results (3)

<table>
<thead>
<tr>
<th>Case</th>
<th>Gram Stain</th>
<th>Bacteria identified</th>
<th>Time to detect</th>
<th>Culture of associated blood products</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>EX201</td>
<td>+ve  <em>Coagulase Negative Staphylococcus</em></td>
<td>5.7 hours</td>
<td>RC (-), FFP (-)</td>
</tr>
<tr>
<td>EX565</td>
<td>-ve</td>
<td><em>Propionibacterium acnes</em></td>
<td>6.5 days</td>
<td>RC (+), FFP (-)</td>
</tr>
<tr>
<td>E1491</td>
<td>-ve</td>
<td><em>Propionibacterium acnes</em></td>
<td>5.5 days</td>
<td>RC (T), FFP (-)</td>
</tr>
<tr>
<td>E2042</td>
<td>-ve</td>
<td><em>Coagulase Negative Staphylococcus</em></td>
<td>10.7 hours</td>
<td>RC (-), FFP (T)</td>
</tr>
</tbody>
</table>
Conclusion(1)

- Bacterial contamination is still present in platelet concentrates at 5 days or 7 days from collection despite routine bacterial surveillance (BST) on day 2.

- The risk bacterial contamination at day 5 or 7 after BST is about 0.133%.

- *Propionibacterium acnes* and *coagulase negative Staphylococcus* are the bacteria identified.

Conclusion(2)

- These bacteria were not identified in the BST culture bottles when incubated for 7 days (because of sampling effect or the original bacterial load being too low).

- *Propionibacterium acnes* is believed to be clinically insignificant because of its slow growing nature and the lacking of report of clinically significant sepsis.

- If *Propionibacterium acnes* is regarded as clinically insignificant, the residual risk will be reduced to 0.033% and 0.066% at day 5 & 7 respectively.
Conclusion(3)

- We therefore cannot justify the application of BST to extend platelet shelf life from 5 to 7 days without additional risk of bacterial contamination.

Pathogen Inactivation/Reduction- Major Concerns

- Platelet Loss up to 30%
  - Processing
  - Chemical Compound

- Killing Capacity:
  - Unable to Kill HAV
  - Log 4 – 5 Bacillus, Pseudomonas, Not Effective Against Bacterial Spores
  - In General log 6 up to 8 for Viruses
    - Viremic Stage: Higher Number of Particles (10^{12} Parvo B19, 10^7-10^8 for HIV – HCV even in the Chronic Infection Stage)
Pathogen Inactivation/Reduction - Major Concerns

- **Cost-Effectiveness**
  - Viral and Bacterial Risk Reduced by Testing
  - Price-Positioning ~ 120 - 100 USD per Product
  - Labour Intensive, Space Occupying

- **Potential Side Effects**
  - Toxicity
  - Mutagenicicity

Thank you for your attention!